

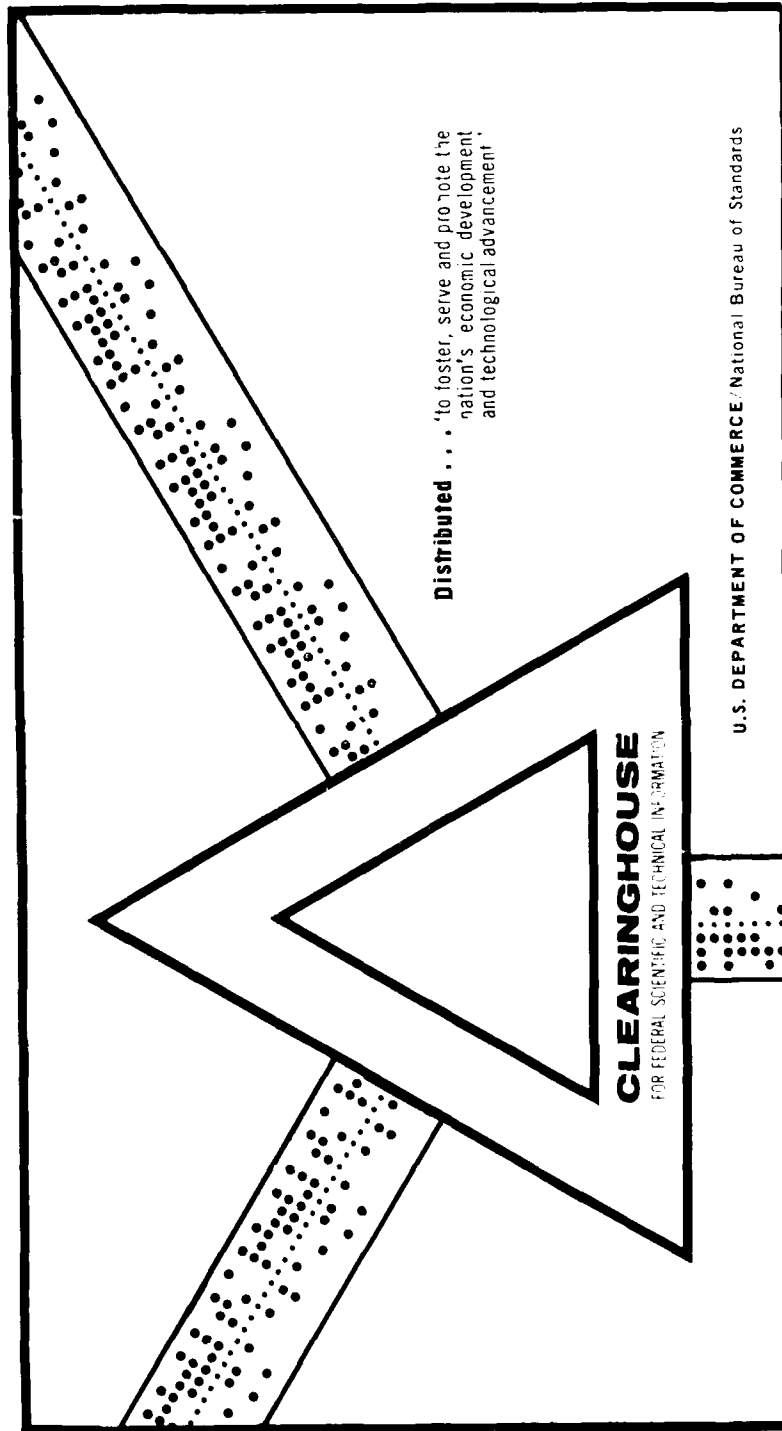
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CONTROL OF MARINE SULFATE --REDUCING BACTERIA IN WATER - DIS-
PLACED SHIPBOARD FUEL STORAGE TANKS

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Naval Research Laboratory
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Control of Marine Sulfate-Reducing Bacteria in Water-Displaced Shipboard Fuel Storage Tanks

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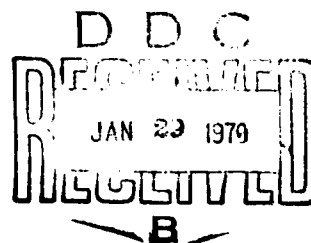
*Marine Biology & Biochemistry Branch
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December 1969



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ABSTRACT

Marine sulfate-reducing bacteria are responsible for a corrosive condition which develops in hydrocarbon fuel storage tanks employing sea water for displacement. Progress toward finding a method of control alternative to the currently used sodium chromate treatment for aviation gasoline tanks on aircraft carriers is reviewed. None of the currently accepted gasoline additives such as corrosion inhibitors and antioxidants are effective against sulfate-reducing bacteria. Increasing the alkalinity of the aqueous phase to a pH of 9 or 10 does not significantly inhibit their growth. Of a number of water soluble biocides which have been evaluated, one commercial compound appears effective at concentrations of a few parts per million. An improved laboratory method of obtaining reproducible inocula of sulfate-reducing organisms is being developed and will be used to carry out further studies of potential growth inhibitors.

PROBLEM STATUS

This is an interim report; work on the problem continues.

AUTHORIZATION

NRL Problem G04-01
Project RR 104-03-41-5503

CONTROL OF MARINE SULFATE-REDUCING BACTERIA IN WATER-DISPLACED SHIPBOARD FUEL STORAGE TANKS

BACKGROUND

For a number of years the Navy has encountered sporadic difficulty with a corrosive condition which develops in hydrocarbon fuel storage tanks on ships and submarines employing sea water for displacement or ballast. The origin of this condition has been traced to contamination by sulfur and sulfur compounds produced through the metabolic reduction of sulfate in the sea water by certain indigenous, anaerobic bacteria (1-8). These compounds are responsible for the development of corrosive or "sour" fuel and for accelerated metal corrosion both in the storage tanks and in the equipment utilizing the fuel. In several notable instances considerable losses in fleet efficiency have been incurred because of the necessity of discarding sour fuel and of removing sludge and scale from tanks.(2,3,9).

Corrosive conditions traceable to sulfate-reducing bacteria have developed under a variety of storage conditions in diesel fuel, jet aircraft fuel (JP-5), and gasoline tanks (3,5-8,10-13). The most acute current problem occurs in aviation gasoline (Avgas) storage tanks aboard aircraft carriers. For this reason efforts at NRL to develop control measures for sulfate-reducing bacteria have been primarily concerned with Avgas/sea water systems. However, progress made on this particular problem is expected to be applicable to other fuels and storage facilities.

The only currently acceptable treatment for controlling sulfate-reducing bacteria in Avgas tanks aboard aircraft carriers is by the addition of sodium chromate to the displacement sea water to give a concentration of 0.10 - 0.15% (3,9,11). This treatment effectively stops the growth of the bacteria and also inhibits metal corrosion. Before applying the sodium chromate treatment, however, it is necessary that the tanks be thoroughly cleaned and suitably lined. Otherwise the chemical action of chromate with sludge or scale in the tank will result

in the production of sulfur or sulfur compounds which sours the fuel. A control method not requiring expensive and time-consuming cleaning and coating operations would be much preferred. Further objections to the use of chromate are that relatively large amounts are necessary to attain the required concentrations in the displacement water and that marine life is likely to be exposed to toxic concentrations of chromate when the treated water is off-loaded during refueling.

A suitable control method for sulfate-reducing bacteria must not only avoid the objections inherent in the chromate treatment but also should not increase corrosion of the storage tanks or affect the quality of the fuel significantly. In general the addition of a fuel-soluble material to the fuel-water system is not desirable since extensive tests would have to be conducted to evaluate its effect on fuel systems, gum formation and engine performance. Additives which promote the emulsification of water in the fuel are also to be avoided.

In addition to these rather stringent restrictions imposed by the fuel storage system, work with sulfate-reducing organisms in the laboratory involves additional difficulties. Cumbersome anaerobic procedures are necessary since these bacteria will not grow or reduce sulfate when oxygen is present; typically, they have low growth rates and are exacting in their growth requirements. It may be surprising that these organisms appear so fastidious when grown in the laboratory even though there is ample evidence of their prolific activity under field conditions. This is a reflection of an inadequate knowledge of the nutrients and physical conditions necessary for optimum growth. There is also a nearly complete absence of information concerning interdependencies which may exist between these bacteria and the other microorganisms with which they are invariably associated in nature.

In spite of a lack of basic information applicable to the Navy's problem we have proceeded with the hope of avoiding time-consuming studies with pure cultures of organisms under conditions bearing little relation to the situation in fuel storage tanks. Accordingly, laboratory test systems have been set up which simulate on a small scale the field conditions. This report is intended to present briefly the more significant

laboratory findings of the past year in approximately chronological order. No firm conclusions can be drawn thus far as to a suitable method of controlling sulfate reducers, but several candidate approaches have been tested and the directions in which solutions should be sought are more narrowly defined.

METHODS

Inocula

Microbial cultures used in the early part of the work reported here came originally from infected displacement water out of shield tanks carrying JP-5 aboard the USS ENTERPRISE (6). The water samples were taken in April 1968 and received at NRL about a month later. No attempt was made to isolate individual species of organisms although the presence of a variety of aerobes as well as anaerobes was demonstrated (5). Growth of sulfate-reducing organisms was obtained in commercially available sealed vials (Difco Laboratories) containing API medium (14) and transfers were made to fresh API vials and to screw-capped 16 x 125 mm test tubes containing Sisler's triple strength medium (15) (Sisler's 3x) overlaid with 1-2 cm. of paraffin oil. Growth was at 26-27°C in the dark. Inoculations for the test system were obtained from organisms grown in these media. The formulations of media are given below:

<u>API</u>		<u>Sisler's 3x</u>	
Sodium lactate (60%)	4.0 ml	Calcium lactate	10.5 g
Yeast extract	1.0 g	Neopeptone	3.0 g
Ascorbic acid	0.1 g	Yeast extract	3.0 g
MgSO ₄ ·7H ₂ O	0.2 g	Ascorbic acid	0.3 g
K ₂ HPO ₄ (anhyd.)	0.01 g	MgSO ₄ ·7H ₂ O	0.6 g
Fe(NH ₄) ₂ (SO ₄) ₂ ·6H ₂ O	0.1 g	K ₂ HPO ₄ (anhyd.)	0.6 g
NaCl	10.0 g	Fe(NH ₄) ₂ (SO ₄) ₂ ·6H ₂ O	0.3 g
Distilled water	1 l	Agar	3.0 g
		Sea water	1 l

The ferrous ammonium sulfate in these media forms a black precipitate in the presence of hydrogen sulfide and thus acts as an indicator for growing sulfate-reducing organisms.

The intent in our procedures has been to employ the mixed species of organisms actually present in the contaminated fuel tanks in order to simulate realistic fleet conditions. Unfortunately, recurring difficulties have been encountered in obtaining consistent growth of the sulfate-reducing organisms in the mixed inocula, even under apparently identical conditions. It was found that active inocula could not be maintained on successive transfers in API vials or in tubes of Sisler's 3x media. Better results were obtained when cultures were either transferred alternately between the two media or when they were transferred continuously on Sisler's 3x with appropriately timed inoculations into API vials for use in experiments. However, these procedures also have occasionally failed to give reproducible inocula of sulfate-reducing bacteria. The trouble may be due to imbalances in the growth of the various competing organisms of the mixed culture under laboratory conditions. Despite these difficulties, the use of a mixed inoculum in the laboratory test system still seems preferable to the artificiality of working with a pure culture of a single species of sulfate-reducing bacteria. Alternative methods of maintaining a consistent inoculum are being tried and are discussed below.

On March 19, 1969, fresh samples of infected displacement sea water and corrosive gasoline from Avgas tanks aboard the USS YORKTOWN were delivered to NRL by personnel of the Naval Fuel Supply Office (13). These samples had been protected from any significant contact with air and were received at NRL within 1-2 days of collection. Copper strip corrosion tests (ASTM Method D130) were made on the fuel samples and the sea water samples were assayed for sulfate-reducing bacteria using API vials. The results, shown in Table 1, confirm the olfactory evidence that considerable amounts of hydrogen sulfide were being produced by microorganisms in the water and that the fuel was sour. That the water samples were heavily contaminated with hydrogen sulfide was further demonstrated by the fact that exposure of a portion of one sample to air overnight resulted in a milky suspension which proved to be due to

colloidal sulfur, produced presumably by the oxidation of hydrogen sulfide. From these water samples an initially very active culture of sulfate-reducing bacteria was obtained and most subsequent work was carried out with inocula obtained from this source.

A comparison of the growths of sulfate-reducing bacteria using old inoculum from the USS ENTERPRISE and new inoculum from the USS YORKTOWN is given in Table 2.

Procedure

For most of the test experiments two-phase systems in 25 x 150 mm test tubes with Teflon-lined caps were used. Usually the aqueous phase consisted of 5 ml of medium and inoculum. The medium customarily used was the API formulation given above. It was made up in the laboratory either with distilled water or with aged sea water filtered through Millipore HA filters. Occasionally filtered sea water alone with the addition of 0.01 per cent ferrous ammonium sulfate as a sulfide indicator was used as the medium. Media were sterilized in an autoclave at 15 lbs. pressure for 15 min. or, in some cases, by filtering through Millipore HA filters.

The aqueous phase was overlaid with 40 ml of fuel or other liquid hydrocarbon. When inhibitors were being evaluated, they were added to the fuel or aqueous phases before introducing the inoculum. Inoculations usually consisted of 0.5 ml of a suspension of the mixed culture removed by syringe from API vials after a growth period of 2-5 days. Incubation of test experiments was at 26-27°C in the dark without agitation. Unless stated otherwise the pH of the medium was 7.5 - 8.2.

RESULTS AND DISCUSSION

Avgas Additives as Microbial Inhibitors:

In early experiments it was found that the sulfate-reducing organisms grew well under each of the following: paraffin oil (Fisher, white, light,

N.E.), refined kerosene (Bayol 35), isooctane, 95% isooctane-5% toluene mixtures, JP-5, diesel fuel (local commercial source), Navy multi-purpose fuel (from USS BIGELOW), and 90% paraffin oil-10% Avgas mixtures. They would not grow under our sample of Avgas, under commercially available gasolines with or without tetraethyl lead, or under a mixture of 10% paraffin oil-90% Avgas. This suggested that one or more of the anti-oxidants or corrosion inhibitors added to Avgas and gasolines might be acting as microbial growth inhibitors. These additives include surfactants and phenolic derivatives, substances which might reasonably be expected to be bactericidal if they enter the water phase even in trace amounts. A number of such materials are approved for use in military fuels (16,17) but there is no readily available way of determining which are present in a particular sample of fuel. We proceeded to test all of the approved types of corrosion inhibitors and antioxidants in order to learn whether one or more of them present in our Avgas samples might be acting as inhibitors of sulfate-reducing activity. Since sulfate-reducing organisms have been demonstrated in Avgas tanks on aircraft carriers, it was assumed that not all additives could be microbial inhibitors or that the physical situation in the tanks could not allow the same opportunity for the inhibitor to enter the aqueous phase as it does under the laboratory conditions. The prospect of finding an inhibitor of microbial activity from among the approved additives for fuel was particularly attractive because such a material could be recommended without further evaluation of its effects on fuel.

In the experiments designed to test this hypothesis, antioxidants and corrosion inhibitors were added to the fuel phase in concentrations as much as three times higher than ordinarily allowed in fuels. The results are summarized in Table 3. Table 4 shows results of tests with Avgas which had been treated in various ways to remove additives, including lead tetraethyl and ethylene dibromide. It can be concluded that anti-oxidants and corrosion inhibitors are not responsible for a significant

inhibition of sulfate-reducing bacteria under the laboratory conditions. Neither does it appear that other additives such as lead tetraethyl and ethylene dibromide are responsible since these would be largely removed by distillation and silica gel adsorption, respectively. Whether some hydrocarbon constituent in Avgas inhibits the growth of this particular species of sulfate-reducing organism or whether some constituent absent from Avgas but present in higher boiling fuels provides a factor necessary for growth is not clear. No further work has been undertaken to decide this question since a lengthy, complex investigation would be required.

When fresh sulfate-reducer inocula were obtained from infected Avgas tanks of the USS YORKTOWN, it was found that growth readily occurred under Avgas in the laboratory system (Table 2). Thus either the earlier culture was not adapted to grow under Avgas (having been obtained originally from JP-5 tanks) or it had lost this ability through successive transfers on laboratory media. When experiments were made with Avgas containing higher than normal amounts of the additives, strong positive growth indications were obtained with the YORKTOWN organisms. This constitutes conclusive proof of the ineffectiveness of these fuel additives to inhibit the growth of sulfate-reducing bacteria in a contiguous water phase.

Control by pH Adjustment:

It has been pointed out by Hazlett (18) that an increase in the pH of an aqueous phase containing hydrogen sulfide would reduce its entrance into a contiguous fuel phase because its ionization as a weak acid would be promoted, thereby favoring its retention in the water. Data were presented showing that fuel in contact with a water phase containing known concentrations of hydrogen sulfide became progressively less corrosive with increases in the pH of the water. Thus, increasing the pH of displacement water to 9.5 to 10.0 by the addition of alkali might prevent fuel from becoming corrosive in spite of the generation of hydrogen sulfide by sulfate-reducing bacteria.

The success of such an approach would depend on whether appreciable elemental sulfur is generated either indirectly by oxidation of hydrogen

sulfide or directly by microbial metabolism. Owing to its extreme insolubility in water, sulfur would, under equilibrium conditions, be almost exclusively distributed in the fuel phase regardless of the pH of the aqueous phase. Wachs, et al (19) have shown a correlation between the presence of elemental sulfur and degree of corrosiveness of fuel stored over small water bottoms in open shore-based tanks infected with sulfate-reducing bacteria. No such data are apparently available for tanks containing large volumes of sea water and no vapor space. Since the question of whether elemental sulfur is generated in fuels stored under the conditions prevailing in shipboard tanks is crucial for the success of control measures using high pH in the aqueous phase, we determined the elemental sulfur content of the Avgas samples received from the USS YORKTOWN using the method of Bartlett and Skoog (20). Qualitative tests for hydrogen sulfide were also made by suspending moist lead acetate paper in the vapor phase above both the Avgas and water samples. The results are included in Table 1. In agreement with Wachs, et al (19), there is a close correlation between corrosiveness and elemental sulfur content of the fuels. The sulfur content did not depend on whether the fuel for analysis was taken from the top or bottom of the sample bottle or from bottles which had been open to the atmosphere or had been closed with little or no access to air since the time of collection from the ship tanks. No indication of hydrogen sulfide above the fuel samples was found but the water samples showed strong positive reactions for hydrogen sulfide, especially those taken from the tanks where the fuel was most corrosive. These data, though inadequate for firm conclusions, do suggest that fuel corrosiveness of an intransient nature in the carrier Avgas tanks is mainly caused by dissolved sulfur, not hydrogen sulfide. Since hydrogen sulfide is a major end product of sulfate reducer metabolism, the sulfur in the fuel arises probably from the oxidation of hydrogen sulfide to sulfur taking place in the fuel or aqueous phases. Thus only if this oxidation occurs mainly in the fuel phase would raising the pH of the water reduce fuel corrosiveness.

Further experimental work will be necessary to resolve this matter.

High pH, in addition to keeping hydrogen sulfide in the aqueous phase, might also inhibit the growth of sulfate-reducing organisms. Experiments were carried out to test this possibility. Table 5 shows the results using the standard laboratory test system. The pH of the aqueous media was increased by the addition of sodium hydroxide. No significant inhibition was obtained up to a pH of 10.

Organic Biocides and Inhibitors:

From a survey of the journal and commercial literature several chemicals which are especially effective in preventing bacterial growth appeared worth evaluating in our system. Results with those which have been tested thus far are shown in Table 6.

Biobor JF, a trade name of U. S. Borax and Chemical Corp. for a mixture of dioxaborinanes, is reported to have been used successfully to inhibit microbial growths in certain diesel and jet fuel systems. It showed no inhibition of sulfate-reducing bacteria in our test system.

Wachs, et al (19) obtained good inhibition of sulfate-reducing bacteria with methyl violet at 10 ppm but we were unsuccessful in obtaining significant inhibition with this dye at 20 ppm or with two other related basic dyes, crystal violet and malachite green. The use of different strains or even different species of organisms may be responsible for the lack of agreement. Ours came from a marine environment whereas theirs were probably of terrestrial origin.

The water soluble anti-icing fuel additive, methyl cellosolve, has been useful in inhibiting microbial growth in aircraft fuel tanks where water bottom accumulations are small. Both methyl and ethyl cellosolve were effective against sulfate-reducing organisms in our test system but such high concentrations are required that they would not be practical to use in the large volumes of water required for displacement in tanks aboard ships.

"Omadine" and "Omacide" are trade names of Olin Matheson Co. for hydroxypyridine thione compounds available as the sodium salt (sodium "Omadine") or in a less pure-hence cheaper-form mixed with sodium chloride and acetate ("Omacide-6"). These materials were effective against sulfate-reducing bacteria at low concentrations especially where sea water alone served as the growth medium. We observed, however, that other organisms in the mixed inoculum which were not sulfate reducers were apparently able to grow in the presence of these compounds. These materials deserve further evaluation.

Chlorine Compounds:

Chlorine compounds are generally accepted as extremely effective biocides. Recent experiments at this Laboratory have demonstrated the usefulness of hypochlorites in killing extremely hardy bacterial spores under a variety of conditions (21). Preliminary experiments have been made to determine the effectiveness of chlorine compounds against sulfate-reducing organisms. Hypochlorite solutions prepared from supertropical bleach and a commercial product, Dearcide 719 (Dearborn Chemical Co.), said to be a stabilized, non-corrosive solution of chlorine dioxide, have been used. Unfortunately the results thus far are not clear cut. The usual ferrous indicator salt cannot be used in the aqueous medium of the test system because it reduces chlorine compounds and in turn is oxidized. This means that the chlorine compounds are inactivated and the hydrogen sulfide indicator action of the ferrous ion is destroyed. Alternative methods for the detection of hydrogen sulfide will be required. If further work shows chlorine compounds to be effective against sulfate reducers, it will have to be determined whether their strong oxidizing action is capable of releasing sulfur compounds from sludges accumulating in fuel storage tanks just as chromate apparently does.

FUTURE PLANS

The recurring difficulty, mentioned above, of obtaining consistent inocula of active sulfate-reducing bacteria in mixed cultures with other organisms from ship Avgas tanks has forced an investigation of other methods of maintaining reproducible inocula for the test system. One approach is to isolate pure cultures of sulfate-reducing organisms under strict anaerobic conditions and then to use subcultures of these as supplements to the usual mixed inocula. Such a procedure has been used in work with sulfate reducers of terrestrial origin to insure the inclusion of active sulfate reducers in every inoculation and might prove useful here (22). In another approach to this problem large volumes of culture medium are being inoculated with mixed populations of organisms taken directly from water samples of infected Avgas tanks under conditions as nearly like those in the tanks as is possible to duplicate in the laboratory. Inocula from such a source might be reproducible over a long enough period of time to allow an extended series of experiments with the small-volume test systems. The results of these and possibly other attempts to obtain reproducible inocula will be presented in a subsequent report.

When reproducible experiments with a laboratory test system are possible, a more thorough evaluation of two of the biocides already mentioned in this report, namely, sodium Omadine and certain chlorine compounds, will be carried out. Studies of several additional anti-microbial agents will also be made, with due heed to the restrictions that these should not affect the properties of the fuel, that they should be easy and safe to apply and not be likely to poison marine life when discharged. It is anticipated that this phase of the work will not involve a large scale screening study of many biocides but that work can be concentrated instead on those relatively few materials, which from the literature or from chemical considerations, are believed to be particularly effective candidates for controlling sulfate-reducing bacteria. When promising biocides are found they will be tested in larger fuel/ocean water systems to simulate more closely the conditions in a carrier tank. These materials will also be evaluated for other

fuel storage systems such as JP-5, diesel fuel, Navy distillate fuel and others where sulfate-reducing organisms can be expected to exist.

REFERENCES

1. Zobell, C.E., Producers Monthly, 22, No.7, (1958) p. 12.
2. Johnson, J.E., NRL Conference Report 6180-209; JEJ:ec, November 9, 1966.
3. Kelley, B.E., NAVSECPHILADIV Report of Travel 10330 (FA-157) January 27, 1967.
4. Hazlett, R.N., NRL Memorandum Report 1779, April 17, 1967.
5. Klemme, Dorothea E., NRL Memorandum Report 1943, November 1968.
6. Collmus, B.A., NFSO, POL Technical Assistance Team Report 44A/Ser 44-18, May 20, 1968.
7. Collmus, B.A., NFSO, POL Technical Assistance Team Report 44A/Ser 44-22, June 11, 1968.
8. Collmus, B.A., NFSO, POL Technical Assistance Team Report 44A/Ser 44-34, October 11, 1968.
9. Hazlett, R.N., NRL Memorandum Report 1737, December 28, 1966.
10. Davis, E.C., NAVSEC, Washington, D.C., private communication.
11. NAVSECPHILADIV 6764/BK, 10340 (FA-185) March 22, 1968.
12. NRL Letter Report 6120-172:DEK:gsu, Ser 3867, April 27, 1967.
13. Collmus, B.A., NFSO, POL Technical Assistance Team Report 441/Ser 44-48, March 25, 1969.
14. Allred, R.C., Producers Monthly 22, No.9, (1958), p.32.
15. Sisler, F.D., and Zobell, C.E., J. Bacteriol. 62, (1951), p.117.
16. Military Specification, "Inhibitor, Corrosion, Fuel Soluble", MIL-I-25017B, October 22, 1962.
17. Military Specification, "Gasoline, Aviation", MIL-G-5572D, 3.3.1, December 22, 1965.
18. Hazlett, R.N., NRL Memorandum Report 1956, January 1969.
19. Wachs, A.M., Bentur, S., Kott, Y., Babitz, M., and Stern, A.B., Ind. Eng. Chem. Process Design Dev. 3, (1964), p.65.
20. Bartlett, J.K, and Skoog, D.A., Anal. Chem. 26, (1954), p. 1008.

21. Fielding, G.H., Echols, W.H., and Neihof, R.A., NRL Report 6506, February 27, 1968.
22. Tuttle, J.H., Dugan, P.R., and Randles, C.I., Appl. Microbiol. 17, (1969), p. 297.

Table 1. Assay For Corrosiveness of Avgas And For Sulfate-Reducing
Bacteria in Displacement Water From Avgas Tanks Of The
USS YORKTOWN

Sample	Tank	Corrosion Index ¹	Sulfur (Elemental) ppm	Hydrogen Sulfide ²	Sulfate- Reducing Bacteria ³
AVGAS Sample 1	Port draw-off	4-A	13	0	-
AVGAS Sample 2	Port draw-off	4-A	13	0	-
AVGAS	Forward port outer	3-B	-	0	-
AVGAS	Forward star- board draw-off	1-B	<1	0	-
Displacement sea water (8 sequential samples)	Forward port outer	-	-	++++ (all samples)	++++ (all samples)

¹Corrosion rated with increasing severity from 1A to 4C (ASTM Method D130).

²"0" indicates no reaction with lead acetate paper after one day, "++++" indicates a positive test within one minute.

³"++++" denotes a strong positive indication in API vials after one day of incubation.

Table 2. Comparison of Growths of Sulfate-Reducing
Bacteria Derived From Different Sources

Fuel	Aqueous Phase	Sulfate-Reducing Bacteria ¹	
		Old inoculum (USS ENTERPRISE)	New inoculum (USS YORKTOWN)
Avgas	API-sea water	0	+++
Avgas	API-distilled water	0	+
Dayol	API-distilled water	++++	++++

¹Estimation of bacterial growth is based on intensity of blackening of the medium from barely perceptible, "+", to dense, "++++"; "0" denotes no growth.

Table 3. Avgas Additives as Inhibitors of
Sulfate-Reducing Bacteria

Additive	Conc. ppm.	Fuel Phase	Aqueous Phase ¹	Sulfate- Reducing Bacteria ²
duPont #22	50	Isooctane-toluene	API-sea water	+
	50	" "	API-dist. water	+
	50	Paraffin oil	" " "	+++ ³
	100	Avgas	API-displ. water	+++ ³
duPont #23	50	Isooctane-toluene	API-sea water	+
	50	" "	API-dist. water	+
	50	Paraffin oil	" " "	+++ ³
	100	Avgas	API-displ. water	+++ ³
duPont #29	100	Avgas	API-displ. water	+++ ³
duPont #30	100	Avgas	API-displ. water	+++ ³
duPont #31	100	Avgas	API-displ. water	+++ ³
2-6-di-t-butyl phenol	50	Isooctane-toluene	API-sea water	+
	50	" "	API-dist. water	+
	50	Paraffin oil	" " "	+++ ³
	100	Avgas	API-displ. water	+++ ³
duPont #2	60	Isooctane-toluene	API-sea water	+
	60	" "	API-dist. water	0
	60	Paraffin oil	" " "	++ ³
	100	Avgas	API-displ. water	+++ ³
duPont AFA-1	60	Isooctane-toluene	API-sea water	+
	60	" "	API-dist. water	+
	60	Paraffin oil	" " "	+++ ³
	100	Avgas	API-displ. water	+++ ³
Lubrizol 541	60	Isooctane-toluene	API-sea water	+
	60	" "	API-dist. water	+
	60	Paraffin oil	" " "	+++ ³
	100	Avgas	API-displ. water	+++ ³
Tolad 244	60	Isooctane-toluene	API-sea water	+
	60	" "	API-dist. water	+
	60	Paraffin oil	" " "	+++ ³
	100	Avgas	API-displ. water	+++ ³
TRI-182	60	Isooctane-toluene	API-sea water	+
	60	" "	API-dist. water	+
	60	Paraffin oil	" " "	+++ ³
	100	Avgas	API-displ. water	+++ ³

¹ In the aqueous phase "dist." is distilled water; "displ." is displacement water.

² Estimation of bacterial growth is based on intensity of blackening of the medium from barely perceptible, "+", to dense, "+++"; "0" denotes no growth.

³ New inoculum cultured from samples of displacement water from USS YORKTOWN Avgas tanks received March 1969; the other inoculations were from cultures obtained from USS ENTERPRISE JP-5 tanks June 1968.

Table 4. Growth of Sulfate-Reducing Bacteria Under
Avgas Treated For Removal of Additives

Avgas Treatment	Aqueous Phase	Sulfate-Reducing Bacteria ¹
Extracted with distilled water	API-sea water	0
Extracted with distilled water and sea water	API-sea water	0
Passed through silica gel	API-sea water	0
Passed through silica gel	API-distilled water	0
Redistilled (to 90%)	API-sea water	0
Redistilled (to 90%)	API-distilled water	0

¹ All inocula were cultured from samples of displacement water from USS ENTERPRISE; "0" denotes no blackening of the medium - hence no growth.

Table 5. Effect of High pH on Growth of
Sulfate-Reducing Bacteria

Fuel Phase	Aqueous Phase	pH	Sulfate-Reducing Bacteria ¹
-----	API-sea water (1:1) ² saturated with MgO	10.0	+++ ³
Paraffin Oil	API-distilled water	7.5	+++ ³
Paraffin Oil	API-distilled water	8.5	+++ ³
Paraffin Oil	API-distilled water	10.0	+++ ³
Avgas	API-sea water	8.2	++++ ⁴
Avgas	API-sea water	8.7	+++ ⁴
Avgas	API-sea water	8.9	+++ ⁴

¹ Estimation of bacterial growth is based on intensity of blackening of the medium from barely perceptible, "+", to dense, "++++".

² In API vials with no fuel phase.

³ Old inoculum from cultures obtained from USS ENTERPRISE.

⁴ New inoculum from cultures obtained from USS YORKTOWN.

Table 6. Effect of Biocides on Growth of
Sulfate-Reducing Bacteria

Additive	Conc. ppm.	Aqueous Phase ¹	Fuel Phase	Sulfate- Reducing ² Bacteria ³
Biobor JF ⁸	250	API-dist. water	Bayol	+++
	500	" " "	"	+++
	1000	" " "	"	++
Methyl violet	10	API-displ. water	Avgas	+++
	20	Sea water	"	+++
Crystal violet	20	Sea water	Avgas	+++
Malachite green	20	Sea water	Avgas	+++
Methyl cellosolve	10 ⁵	Sea water	Avgas	0
Ethyl cellosolve	10 ⁵	Sea water	Avgas	0
Omacide 6 ⁴	5	Sea water	Avgas	±
	5	API-sea water	"	±
	10	Sea water	"	0
	20	Sea water	"	0
	20	API-sea water	"	±
	30	Sea water	"	0
Sodium Omadine ⁴	5	Sea water	Avgas	±
	5	API-sea water	"	±
	20	Sea water	"	0
	20	API-sea water	"	±
	30	Sea water	"	0
	30	API-sea water	"	0
	30	API-dist. water	"	0
Sodium Omadine ⁴ + Borax (1:50)	1	Sea water	Avgas	±
	1	API-sea water	"	++
	5	Sea water	"	±
	5	API-sea water	"	±

¹In the aqueous phase "dist." is distilled water; "displ." is displacement water.

²Estimation of bacterial growth is based on intensity of blackening of the medium from barely perceptible, "+", to dense, "++++"; "0" denotes no growth; "±" denotes a slight grey precipitate - hence questionable growth.

³Dissolved in fuel phase; all other additives were dissolved in the aqueous phase.

⁴Concentrations are all in terms of the active ingredient, sodium omadine, and are thus directly comparable.

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13. ABSTRACT <p>Marine sulfate-reducing bacteria are responsible for a corrosive condition which develops in hydrocarbon fuel storage tanks employing sea water for displacement. Progress toward finding a method of control alternative to the currently used sodium chromate treatment for aviation gasoline tanks on aircraft carriers is reviewed. None of the currently accepted gasoline additives such as corrosion inhibitors and antioxidants are effective against sulfate-reducing bacteria. Increasing the alkalinity of the aqueous phase to a pH of 9 or 10 does not significantly inhibit their growth. Of a number of water soluble biocides which have been evaluated, one commercial compound appears effective at concentrations of a few parts per million. An improved laboratory method of obtaining reproducible inocula of sulfate-reducing organisms is being developed and will be used to carry out further studies of potential growth inhibitors.</p>		

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Shipboard fuel tanks						
Aviation gasoline						
Hydrocarbon fuels						
Control of microorganisms in fuels						